Journal of Chromatography B, 964 (2014) 50–64

Journal of Chromatography B

journal homepage: www.elsevier.com/locate/chromb

A review of analytical methods for eicosanoids in brain tissue☆

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ARTICLE INFO

Article history:
Received 11 November 2013
Received in revised form 26 February 2014
Accepted 5 March 2014
Available online 14 March 2014

Keywords:
Eicosanoids
Brain tissue
Ultra high pressure liquid chromatography–mass spectrometry
High pressure liquid chromatography–mass spectrometry
Gas chromatography–mass spectrometry
Tandem mass spectrometry

ABSTRACT

Eicosanoids are potent lipid mediators of inflammation and are known to play an important role in numerous pathophysiological processes. Furthermore, inflammation has been proven to be a mediator of diseases such as hypertension, atherosclerosis, Alzheimer's disease, cancer and rheumatoid arthritis. Hence, these lipid mediators have gained significant attention in recent years. This review focuses on chromatographic and mass spectrometric methods that have been used to analyze arachidonic acid and its metabolites in brain tissue. Recently published analytical methods such as LC–MS/MS and GC–MS/MS are discussed and compared in terms of limit of quantitation and sample preparation procedures, including solid phase extraction and derivatization. Analytical challenges are also highlighted.

1. Introduction

Eicosanoids are specific biomarkers of inflammation. Their biosynthesis from polyunsaturated fatty acids can be catalyzed by cyclooxygenase (COX-2), lipoxygenases (LOX), and cytochrome P450 enzymes. Depending on the mechanism/pathway of biosynthesis and parent molecule, different classes of eicosanoids are defined. Brain lipids such as neuroprostanes are produced by peroxidation of docosahexaenoic acid. These lipids are different from eicosanoids and will not be the subject of this review. This article focuses on eicosanoids from Arachidonic Acid (AA).

Arachidonic acid (ϖ-6 polyunsaturated fatty acid) can be metabolized to hydroxyeicosatetraenoic acids (HETEs), dihydroxyeicosatetraenoic acids (DiHETEs), epoxyeicosatreinoic acids (EETs), prostaglandins (PGs) and thromboxane (TX) (Fig. 1). These endogenous eicosanoids are present in small levels in biological fluids and tissues including the brain. In the brain, these markers are important to maintain homeostasis and normal functions such as synaptic plasticity related to long-term depression [1] and protecting cortical neurons against glutamate toxicity [2]. On the other hand, alterations in the levels of these lipids in the brain have been associated with numerous diseases such as Alzheimer's, Parkinson's, Multiple sclerosis, schizophrenia, and epilepsy [3]. The past decade has produced numerous excellent reviews describing the metabolism of these lipid molecules and their role in diseases [4–7].

1.1. Cycloxygenase enzymes, prostaglandins and brain

Cyclooxygenase enzyme is present in the human body in the form of three isozymes: COX-1, COX-2 and COX-3. COX-1 is thought to be only responsible for maintaining homeostasis in numerous physiological functions in the body [8]. COX-3, which was discovered only in 2003, is considered to be an intron-splice variant of COX-1 [9]. On the other hand, COX-2 is involved in numerous inflammatory processes [9]. Both COX-1 and COX-2 are expressed in brain tissue [10]. Additionally COX-1 and COX-2 enzymes can catalyze the reaction that converts arachidonic acid to a stable hydroxyendoperoxide (PGH_2) [11,12]. PGH_2 is then converted into primary prostanoids by various enzymes [11,12]. Prostanoids can be classified into prostaglandins (PGE_2, PGF_2 and PGD_2), prostacyclins (PGI_2) and thromboxanes (TXA_2). PGD_2 is the most abundant prostaglandin synthesized in the central nervous system. It not only regulates functions like temperature and sleep [13] but it also protects the brain from excitotoxic injury [14]. PGE_2 is involved in brain maturation and in regulation of synaptic activity and plasticity [15]. Furthermore being one of the most abundant prostaglandins, PGE_2 is involved in processes leading to classic signs of inflammation

☆ This paper is part of the special issues ACIDS edited by Alexander A. ZOERNER and Dimitrios TSIKAS IG002157 Special Issue: Analysis of Acids/ZOERNER.

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http://dx.doi.org/10.1016/j.jchromb.2014.03.007

Nomenclature

AA Arachidonic acid
BHT Butylated hydroxyl toluene
CID Collision-induced dissociation
CLASS Comprehensive lipidomic analysis by separation simplification
COX Cyclooxygenase
CSF Cerebral spinal fluid
CV Coefficient of variance
CYP-450 Cytochrome P450
CyPGs Cyclopentenone prostaglandins
DiHETEs Dihydroxyeicosatetraenoic acids
DiHETrEs Dihydroxyeicosatreinoic acids
DTPA Diethylenetriaminepenta-acetic acid
ECNCI Electron capture negative chemical ionization
EDTA Ethylenediaminetetra-acetic acid
EETs Epoxyeicosatrienoic acids
ESI Electrospray ionization
ESI-LC-MS/MS Electrospray ionization coupled to high pressure liquid chromatography with tandem mass spectrometry
fmol Femtomol
GC Gas chromatography
GC-FID Gas chromatography-flame ionization detection
GC–MS Gas chromatography–mass spectrometry
GC–MS/MS Gas chromatography–tandem mass spectrometry
GC–MS/NICI Negative ion chemical ionization coupled to gas chromatography–mass spectrometry
HESI Heated electrospray ionization
HETEs Hydroxyeicosatetraenoic acids
HETP Height equivalent theoretical plates
HILIC Hydrophilic interaction chromatography
HPETEs Hydroxyperoxyeicosatetraenoic acid
HPLC High pressure liquid chromatography
HPLC-UV High pressure liquid chromatography-ultraviolet detector
LC Liquid chromatography
LC–MS Liquid chromatography–mass spectrometry
LC–MS/MS Liquid chromatography–tandem mass spectrometry
LOD Limit of detection
LOQ Limit of quantitation
LOX Lipoxygenases
LTs Leukotrienes
MRM Multiple reaction monitoring
MS Mass spectrometry
MS/MS Tandem mass spectrometry
NE-OTf 2-(2,3-naphthalimino)ethyl-trifluoromethanesulphonate
NICI Negative ion chemical ionization
NP-HPLC Normal-phase high pressure liquid chromatography
ODS-silica Octadecylsilyl-silica
PCA Principle component analysis
PFB Pentafluorobenzyl
pg Picogram
PGH_2 Hydroxyendoperoxide
PGs Prostaglandins
Q1 Quadrupole 1
Q3 Quadrupole 3
Q-TOF Quadrupole time-of-tlight mass spectrometer
QTRAP Quadrupole-linear ion trap
RP-HPLC Reverse-phase high pressure liquid chromatography
SAH Subarachnoid hemorrhage
sEH Soluble epoxide hydrolase
SIM Selective ion monitoring
sMRM Scheduled multiple reaction monitoring
SPE Solid phase extraction
SRM Selected reaction monitoring
SV Symptomatic cerebral vasospasm
TBI Traumatic brain injury
TLC Thin layer chromatography
TOF Time-of-flight
TX Thromboxane
UPLC Ultra high pressure liquid chromatography
UPLC–MS/MS Ultra high pressure liquid chromatography–tandem mass spectrometry
UV Ultraviolet

and pain [16]. PGF_2 plays an important role in brain injury and pain [16]. PGI_2 and TXA_2 are potent vasodilators and vasoconstrictors respectively.

1.2. Lipoxygenase enzymes, leukotrienes and the brain

Lipoxygenease are a group of enzymes that catalyzes the reaction, which involves the addition of oxygen to AA producing hydroxyperoxyeicosatetraenoic acid (HPETEs) [17,18]. HPETE then reduces to give leukotrienes (LTs) and hydroxyeicosatetraenoic acid (HETEs) [19]. Cysteinyl leukotrienes, which include LTC4, LTD4 and LTE4, are found to alter cerebral vessel functions [20] and disrupt the blood brain barrier [20]. Moreover, they have been related to brain edema formation [20]. They are also produced in response to numerous acute brain injuries [21]. LTB4 may also be involved in the pathogenesis of ischemic brain edema [22]. Another role LTB_4 plays in inflammation is attracting leukocytes [97]. Inflammation as a result of alterations in blood flow and vascular permeability can be described by the actions of LTC_4 and LTD_4 [97]. Leukotrienes can be involved in inflammatory diseases such as asthma. HETEs are potent vasoactive agents and are altered in cerebrovascular pathologies [22].

1.3. Cytochrome P450 enzymes (CYP-450), DiHETEs, HETEs and the brain

AA undergoes metabolism by CYP-450 to give epoxyeicosatrienoic acid (EETs). CYP enzyme produces four regioisomers of EET from AA: 5,6-, 8,9-, 11,12-, and 14,15-EET [23]. EETs are quickly metabolized in the presence of soluble epoxide hydrolase (sEH) to the corresponding inactive diols, the DiHETEs [24,25]. In the brain, EETs are involved in controlling the cerebral blood flow (CBF) [26]. They are neuroprotective agents because of their anti-inflammatory and anti-thrombotic effects [26]. Zhang et al. found that deletion of sEH, the enzyme that metabolizes EETs to DiHETEs, is protective against ischemic brain injury [27].

2. Quantification of eicosanoids

Currently, immunoassays [28], GC–MS [29], LC–MS [30] and LC–MS/MS [31,32] have been used to analyze eicosanoids. For a long time, immunoassays, (enzyme-linked immunoassays and radio-labeled immunoassays) were considered to be the standard method for lipid analysis. Though these assays were sensitive, they suffered from reproducibility and reduced specificity. A regular

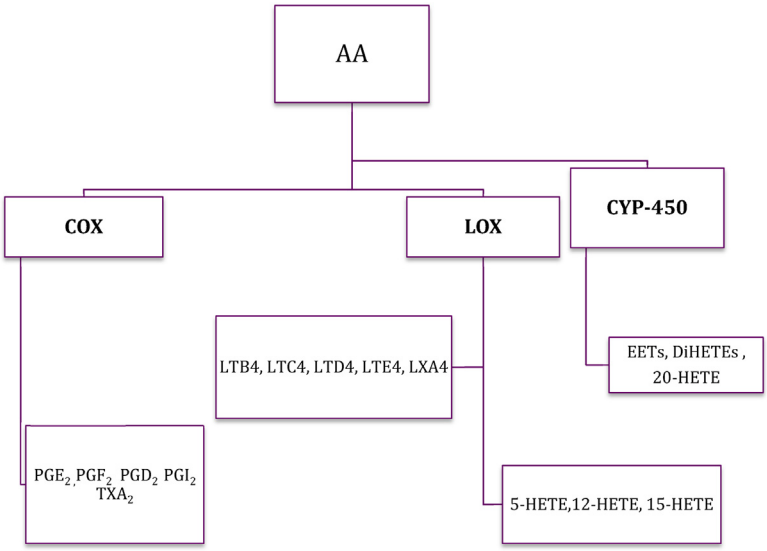

Fig. 1. Oxidative pathway of arachidonic acid by COX, LOX and CYP 450 enzymes.

analysis using HPLC with UV detection is difficult, as most of these lipid molecules do not have good chromophores. GC–MS and GC–MS/MS in negative ion chemical ionization mode have been successfully used to analyze some of these markers. The disadvantage is that, to make it suitable for labile compounds like the EETs, the analyte needs to undergo purification followed by derivatization. Similarly HPLC coupled to fluorescent detectors require these compounds to be derivatized to a complex that fluoresces, as these compounds contain no aromatic or naturally fluorescing systems [33]. Derivatization makes the analysis laborious, expensive and time consuming. The more popular choice when it comes to detection of these biomarkers is LC–MS. One of the reasons is that mass spectrometry (MS) allows quantification at very low levels in complex matrices. Moreover, mass spectrometry gives better isomer separation when compared to HPLC-UV and immunoassays.

In regards to analysis of the brain tissue, the two most important steps are extraction and sample preparation. These are important, as the complex brain matrix produces high chemical background noise, thus reducing selectivity and sensitivity. This review focuses on the various strategies that have been used to analyze eicosanoids in brain in recent years.

2.1. Sample preparation and extraction procedures

Analyzing eicosanoids from biological samples requires extraction and purification of the analyte in order to ensure the analyte is present and free of any interfering impurities. The amount of analyte present depends upon the sample matrix. In various matrices such as the brain, arachidonic acid metabolites exist in extremely low concentrations and extraction is required to increase specificity and sensitivity [34]. Eicosanoids can be extracted using organic solvent (liquid/liquid) or solid phase extraction (SPE). In choosing an extraction method it is important to consider that the method is fast, reproducible and cost effective. The eicosanoids must not be altered by the extraction procedure; therefore, extremes of pH must be avoided [41]. Nevertheless, adjusting the pH can increase efficiency. Signal suppression is a significant problem in analysis of biological samples. Common tissue extraction procedures are complicated by the control of matrix effects and differences in physiochemical properties of analytes [56]. Since eicosanoids precursors are unstable in the matrix, new eicosanoids may be generated during the extraction process. Crucial functions to the extraction process such as homogenization can activate eicosanoid synthesis; therefore, inhibitors and other drugs such as indomethacin, ethylenediaminetetra-acetic acid (EDTA), diethylenetriaminepenta-acetic acid (DTPA), and butylated hydroxyl toluene (BHT) are incorporated into the extraction process to limit eicosanoid formation from the extraction [61].

Many methods are reported in the literature for the extraction of eicosanoids from tissues, most employ the Folch or Bligh and Dyer extraction protocols with modifications [35,36]. The Folch procedure uses chloroform/methanol in a 2:1 volume ratio at 20 times the amount of sample [35]. Partitioning of the two layers can be achieved using a saline solution of ¼ the total volume added. This will also attain a lower layer consisting of all lipids and an upper layer consisting of contaminants. In the end a solution composed of the ratio 8:4:3 chloroform/methanol/water should be utilized to affirm lipid separation into the chloroform layer. Bligh and Dyer adapted the procedure set forth by Folch and improved the amount of solvent used as well as decreased the time of extraction and analysis. In the adapted method 3 mL of 2:1 methanol/chloroform was used per gram or mL of sample. Following mixing, 1 mL chloroform and 1.8 mL water were added to partition the solution into two phases. Using chloroform to re-extract the tissue improves the yield of the lipids. Depending on the desired form of analysis, methods may employ the Folch [63–65] or Bligh and Dyer procedures for extraction of eicosanoids; however, if high sensitivity is required as in mass spectrometry, further purification may be necessary. Some liquid/liquid extractions that are more specific for brain eicosanoids include hexane/2-propanol [37,59,60], ether [38], acetone/chloroform [39,40]. Saunders and Horrocks exhibited a 12–37% increase in recovery of prostaglandins from bovine brain using hexane/isopropanol as compared to the Folch procedure [37]. Acidification of the lower layer allows the eicosanoids to be in a non-ionized form and can be extracted into organic solvents. Caution must be taken during this step as excess acidification can lead to eicosanoid alterations [40]. Tajima et al. modified the Bligh and Dyer procedure for analysis of brain tissue for Alzheimer's disease, in which the liquid/liquid extraction is further purified via SPE [50]. Similarly, Axelsen and Murphy execute a modified procedure of Bligh and dyer for extraction of arachidonic acid in mouse brains to study neurogenerative disorders [51]. In a recent study, extractions of prostaglandins from brain samples were assessed and compared to achieve the best recoveries and lowest detection limits [39]. Golovko et al. explored brain extraction of PGE_2, PGD_2, TXB_2, $PGF_{2\alpha}$ and 6-oxo-$PGF_{1\alpha}$ with methanol followed by

SPE, diethyl ether, hexane/2-propanol and with acetone. Acetone extraction followed by liquid/liquid extraction presented the lowest background noise thus increasing the sensitivity the LC–MS/MS analysis [39,49]. An increase in sensitivity is attributed to the purification of the prostaglandins by acetone. Liquid/liquid extractions require a large amount of solvent, which increases cost of analysis as well as decreases the extraction efficiency and mass spectrometry sensitivity [38]. Brose et al. challenged these issues using a one step methanol extraction, consequently increasing efficiency and sensitivity and decreasing analysis time [42]. Substitution of methanol for acetone/chloroform exhibited superior performance over other liquid/liquid extractions and SPE's. The use of methanol rather than acetone/chloroform improved the recovery of internal standards to (96.7 ± 9.9%), which is a 20% increase over previously reported multiple step liquid/liquid extractions [42]. A concern in using the methanol procedure to extract prostaglandins and isoprostanes from brain tissue is that the methanol extracts will contain hydrophobic lipids that are not eluted from the column during analysis and could therefore overtime shift the retention time of other components by binding to the column [42]. Likewise, Yasaka et al. used methanol to extract arachidonic acid from mouse cerebrum. The tissue was homogenized and centrifuged three times in methanol to increase the extraction efficiency and extrude as many contaminants as possible [44]. A 95% recovery was consistently reported for the extraction of arachidonic acid by Yasaka et al. Urban et al. evaluated the advantages and shortcomings of four extraction solvents for extraction of prostaglandins from brain tissue; ethanol/dichloromethane (1:1), ethanol/10 mM phosphate buffer (85:15), methanol/10 mM phosphate buffer (85:15) and 10 mM phosphate buffer [56]. Using ethanol/10 mM phosphate buffer as the extraction solvent, the prostaglandins demonstrated the most reproducible results, with a coefficient of variance <15% in comparison to ethanol/dichloromethane (<20%), methanol/10 mM phosphate buffer (<25%) and 10 mM phosphate buffer (<30%).

In addition to the large solvent volumes required, other disadvantages of liquid/liquid extractions are that they are time consuming and potential error is introduced with each addition of solvent. To lower the amount of solvent consumption, decrease sample analysis time, lower the cost per sample and increase consistency, SPE's have increased in popularity. SPE concentrates the analyte, thus increasing the sensitivity as well as improving detection limits. Additionally, it removes interfering compounds and impurities, thus protecting analytical systems and increasing efficiency. Higher and more reproducible recoveries are experienced as a result of cleaner extracts as well as tunable selectivity, i.e. choice of solvents for elution. SPE cartridges come in reverse phase (C18), normal phase (silica) and ion exchange (anion or cation). The basic principle behind reverse-phase SPE is that the aliphatic moieties in the lipids can interact with the non-polar stationary phases; consequently, allowing them to be retained and separated from impurities. Various solvents can be used to concentrate the analyte by releasing non-lipid contaminants. Finally the sample can be released by washing the column with a non-polar solvent. SPE is similar to HPLC and differs mainly in the packing of the columns; SPE columns use large silica particles in comparison to micron particle sized HPLC columns. An added advantage of SPE is that it can be combined directly with LC, thus eliminating sample loss and also offering the potential for a fully automated system. A simple procedure to extract eicosanoids from biological tissues is demonstrated by Powell [34]. Eicosanoids are extracted on a hydrophilic stationary phase containing octadecylsilyl-silica (ODS-silica). Samples are loaded onto the column with a 15% methanol solution and washed with 15% methanol, water, petroleum ether and finally methyl formate to elute the eicosanoids [34]. For the extraction of prostaglandins the percent of methanol solution should be lowered to <10% to yield the highest recoveries. In contrast, Powell demonstrated that the extraction of unmetabolized arachidonic acid requires a methanol solution of 30% to accomplish recoveries >80%. In our group, Yue et al. extracted endogenous eicosanoid metabolites in rat brain using an Oasis® HLB SPE cartridge (Fig. 2) eluting analytes with acetonitrile and ethyl acetate and evaporated the remaining solvent under argon [33,43]. The extraction efficiency and the %RSD for PG's, DiHETrE's, HETE's, EET's and AA was 72.13–99.56% and 1.93–14.73% respectively, indicating a reproducible extraction and a reliable bioanalytical method [43]. This extraction procedure reported interference of the components of the SPE with $PGF_{2\alpha}$ and 15-HETE. Similarly, Masoodi and Nicolaou performed an extraction of 27 prostanoids including prostaglandins, prostacyclines, thromboxanes, dihydroprostaglandins and isoprostanes from brain samples on C-18 Phenomenex SPE cartridge, eluting analytes with methyl formate and achieved percent recoveries of 84–110% [31].

Brain tissue has high moisture and fat content [33]. Therefore, since eicosanoids are lipophilic and will bind to fats, choice of solvent during an extraction is vital. For SPE of eicosanoids from brain tissue, solvents must be chosen that dissolve eicosanoids, permeate through brain tissue matrix, break down the tissues and release the eicosanoids and finally induce protein precipitation [33]. Acidic conditions can be used to reduce protein binding as well as warrant the free carboxylic acid form of eicosanoids. In

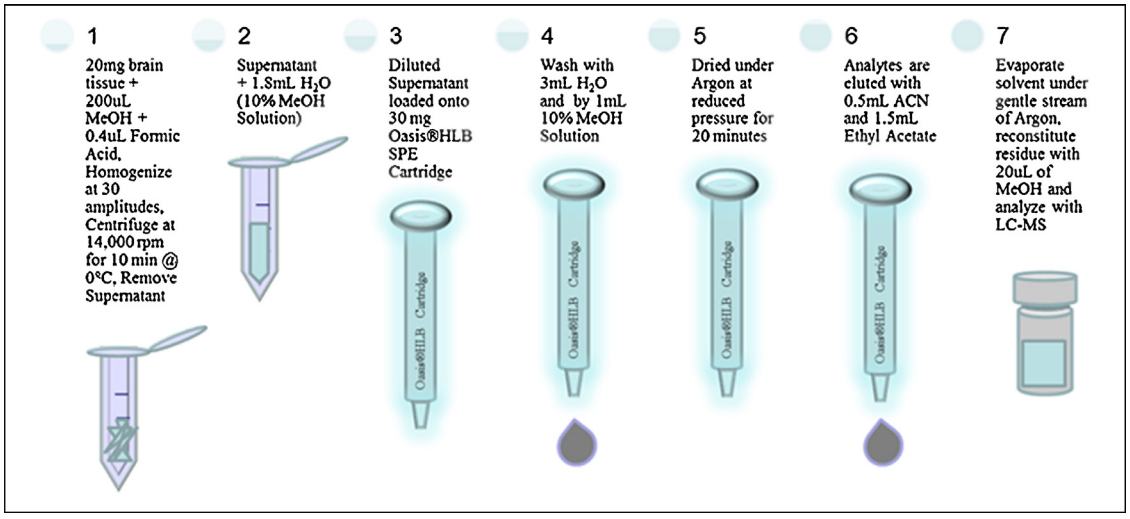

Fig. 2. Yue et al. extraction of eicosanoid metabolites from rat brain.

our group, Yue et al. established that methanol and formic acid should be used for SPE, as they are easily evaporated and compatible with the system. Additionally, the choice of SPE cartridge was evaluated and Oasis® HLB cartridges were chosen as they yielded reproducible results in wet and dry conditions. Many other eicosanoid extraction procedures that also make use of SPE have been reported [30,45–48,52–54,57,58,62,66]. Miller et al. extracted 20-HETE, 15-HETE, 12-HETE, 14,15-EET, 11,12-EET, 8,9-EET, 14,15-DiHETrE, 11,12-DiHETrE, 8,9-DiHETrE and 5,6-DiHETrE metabolites of arachidonic acid in rat brain cortical tissue samples and achieved recoveries ranging from 73% to 94% as well as a %RSD of less than 16.75% [52]. A few disadvantages of SPE are that it can be time consuming depending upon the procedure utilized, SPE cartridges are expensive and for unstable eicosanoids a lengthy extraction process could be detrimental to further precise analysis of the metabolites.

In addition to the extraction procedure, care must be taken when sampling the brain to ensure there is no post-mortem PG synthesis. Brain PG mass is reported 10–40-fold lower using focused microwave irradiation as compared to decapitation [39,95,96]. In addition, PG's can be synthesized during the extraction, which can contribute to higher and greater variability of PG mass found in samples [39]. Golovko and Murphy established microwave irradiation at 70–80 °C as an effective technique to heat-denature enzymes in order to prevent post-mortem PG synthesis [39]. A concern with microwave irradiation is related to PG heat-destruction or trapping denatured proteins. Golovko and Murphy illustrate that PG levels between microwaved and non-microwaved brains (at maximal PG formation) indicating PG's aren't trapped during the microwave process and therefore recovery is not affected [39]. Microwave irradiation prevents dramatic post-mortem induction in brain eicosanoids and allows for measurement of true eicosanoid levels.

Automated methods have been developed that exemplify comparable precision and accuracy to manual operations, while increasing the throughput of sample analysis [55]. A problem in sample preparation often occurs in transfer of materials from the extraction to method of analysis (i.e. GC, LC). Eliminating human transfer of these materials will increase efficiency and decrease sample loss. Advances in technology will allow for more of these types of methods.

2.2. Separation and detection

2.2.1. Gas chromatography–mass spectrometry

Gas chromatography–mass spectrometry (GC–MS) was for a long time a routine analytical technique for quantitation and structure interpretation of eicosanoids; however, because of the cost of equipment and difficulty in sample preparation, fewer laboratories utilize GC–MS. For GC analysis, a molecule must be volatile and thermally stable, which are not properties that all eicosanoids express. As a result, for analysis of eicosanoids derivatization of carboxyl and hydroxyl groups is required to increase their volatility [41]. Following a derivatization step, many analytes can be detected simultaneously. Some examples of derivatization methods are N-acylation, methoximine formation, esterification and trimethylsilyl ether formation [41]. GC-Flame Ionization Detection (FID) is not frequently used for quantitative analysis of eicosanoids; nevertheless, Rosenberger et al. quantified prostaglandin methyl esters using a GC (Trace 2000, ThermoFinnigan, Houston, TX, USA) equipped with a capillary column (SP 2330; 30 m × 0.32 mm i.d., Supelco, Bellefonte, PA, USA) and a FID to determine that rat brain arachidonic acid metabolism increases by infusion of bacterial lipopolysaccharide [84]. The limit of quantitation (LOQ) was not reported but control samples of $PGF_{1\alpha}$, $PGF_{2\alpha}$, TXB_2, PGD_2 and PGE_2 demonstrated linearity to approximately 20 pmol/g of brain sample. FID does not demonstrate information concerning the mass of metabolites, making structure elucidation unreasonable.

Precise quantitation using GC-FID is contingent upon resolution of chromatographic peaks, which is not always possible. Instead, GC–MS is more frequently used as it allows detection of eicosanoids down to picogram (pg) levels in biological samples. GC–MS requires purification steps following derivatization to void any impurities formed during the derivatization process. Common sample matrices analyzed by GC–MS are urine and plasma. Still, methods have been reported for analysis of brain samples by GC–MS. Ogorochi et al. determined the *in situ* contents of PGD_2, PGE_2, $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$ in regions of post-mortem human brain [58]. This method challenges the notion that the concentration of $PGF_{2\alpha}$ is the most abundant prostaglandin in post-mortem brain samples, signifying its potential use as a biological indicator in neural and glial cells as reported by Abdel-Halim et al. [89]. Ogorochi et al. establish that the concentrations of PGD_2, PGE_2 and $PGF_{2\alpha}$ are similar in post-mortem human brain samples and that Abdel-Halim et al. reported values consistent with *in vitro* artificial production of prostaglandins [58]. Distribution of the metabolites in post-mortem human brain samples can be seen in [58] with concentrations ranging from 0.06 to 41.3 ng/g wet weight. In a slightly different scope metabolites of arachidonic acid in mice brain samples were separated by normal-phase high pressure liquid chromatography (NP-HPLC) and the structures of the identified metabolites were confirmed by GC–MS by Amruthesh et al. [75]. Methyl ether methoxylaminetrimethysilyl ether derivatives of 5-HETE, 12-HETE and 15-HETE were assessed using electron impact ionization MS and pentafluorobenzyl (PFB) ester derivatives of 5,6-EET and 14,15-EET were analyzed using negative ion chemical ionization MS. GC–MS is frequently used as an analytical support technique for confirmation of structures separated by other chromatographic techniques. Yang et al. demonstrated a significant increase in arachidonic acid in the brain 24 h after a traumatic brain injury (TBI) using GC–MS and principle component analysis (PCA) to compare serum samples of TBI and normal rats [77].

Specific ionization techniques such as electron capture negative chemical ionization (ECNCI) are commonly used for the detection of eicosanoids following separation by GC. In negative ion chemical ionization (NICI) a carboxylate anion is observed in eicosanoid analysis. A general method for analysis of arachidonic acid in various sections of mouse brain tissue using NICI coupled to GC–MS is demonstrated in [51]. Levels of arachidonic acid in various lipid species were reported from 78 to 492 nmol/g tissue. Wiswedel et al. quantitated monohydroxyeicosatetraenoic acids (HETEs) and F_2-isoprostanes by GC–MS/NICI to study lipid peroxidation in rat brain mitochondria [79]. After hydrogenation and SPE, PFB ester and trimethylsilyl ether derivatives were formed for analysis of HETEs and F_2-isoprostanes by GC–MS/NICI. Quantitative analysis obtained in selective ion monitoring (SIM) mode, monitoring the carboxylate anion $[M-181]^-$ at m/z 399 for the HETEs reported the sum of the HETEs (5-HETE, 8-HETE, 12-HETE and 15-HETE) content in the rat brain mitochondria as 220 ± 40 pmol/mg protein [79]. Additionally, monitoring the carboxylate anion $[M-181]^-$ at m/z 569, the content of F_2-isoprostanes (8-iso-$PGF_{2\alpha}$, 9α,11β-$PGF_{2\alpha}$ and 9α,11α-$PGF_{2\alpha}$) was 0.21–5.4 pmol/mg protein [79]. SIM requires careful extraction and purification to ensure no interference from impurities developed throughout the process. Although not brain analysis, Nithipatikom et al. used GC–MS/NICI to analyze the release of epoxyeicosatrienoic acids (EETs) (5,6-EET, 8,9-EET, 11,12-EET and 14,15-EETs), consequent dihydroxyeicosatrienoic acids (DiHETrEs) and hydroxyeicosatetraenoic acids (HETEs) (19-HETE and 20-HETE) into the coronary venous plasma during coronary artery occlusion and reperfusion in anesthetized dogs [80]. After extraction, pentafluorobenzyl ester and pentafluorobenzyl ester/trimethylsilyl ether derivatives of EETs, DiHETrEs and 20-HETE respectively were formed and detected by SIM at the appropriate m/z. The EETs coeluted and therefore the limit of detection (LOD) is a sum of

the total EETs. The limit of detection was 5, 40 and 15 pg for the EETs, DiHETrEs and 20-HETE respectively [80]. Kadiiska et al. describe that the measurement of isoprostanes, specifically 8-iso-$PGF_{2\alpha}$ by GC–MS/NICI is the most accurate approach for analysis of oxidative stress [90]. In conjunction with that discovery, Milatovic and Aschner demonstrated a procedure using GC–MS/NICI for the measurement of F_2-isoprostanes as markers of oxidative stress in neuronal tissue, reporting the lower limit of detection for F_2-isoprostanes to be in the low picogram range [81]. Tandem mass spectrometry (MS/MS) instruments have gained interest recently in coupling with various chromatography techniques. There are two modes of operation a MS/MS instrument can use, scanning mode and selected reaction monitoring (SRM) or multiple reaction monitoring (MRM) mode. Scanning mode can be performed in the first or second analyzer, in which a specified range of masses are studied. In MRM mode the collision-induced dissociation (CID) of precursor ions produces product ions, which increases the specificity of eicosanoid analysis [78]. Tandem MS instruments increase sensitivity by monitoring the selected reaction rather than the selected ion. An excellent review is written by Tsikas, thoroughly discussing the use of GC–MS and GC–MS/MS for the analysis of the metabolites of arachidonic acid for most biological samples, except the brain [76].

2.2.2. Liquid chromatography

A survey evaluating liquid chromatography (LC) trends from the previous decade indicates that reverse-phase high pressure liquid chromatography (RP-HPLC) is the dominant LC analysis technique [71]. Previously thin layer chromatography (TLC) was employed to separate eicosanoids; however, this technique required standards to be available, which may not exist for endogenous eicosanoids. As compared to TLC, HPLC allows for better separation as a result of a larger number of theoretical plates. Separation efficiency is determined by the particle size, pore size, surface area, stationary phase and the chemistry of the substrate surface [82]. Additionally because HPLC is a nondestructive technique, it can be used for purification purposes [72].

As a consequence of the isomeric and isobaric (ions with the same mass) nature of some of the metabolites of arachidonic acid, chromatography is an essential step in quantification. Various modes can be used to separate the metabolites of arachidonic acid, RP-HPLC, NP-HPLC, chiral HPLC and hydrophilic interaction chromatography (HILIC). Separation in RP-HPLC relies on the hydrophobic properties of the analytes and therefore remains the workhorse for separation of these metabolites. Studying the metabolism of arachidonic acid in mouse brain microsomes, Qu et al. were unable to separate 19-HETE and 20-HETE with RP-HPLC and employed NP-HPLC to determine the percentage of each, 19% and 45% respectively in the sample [73]. NP-HPLC is useful in separating various classes of metabolites. NP-HPLC is often used with chiral columns, improving the separation of enantiomers and regioisomers [74]. Determining the ratio of PGE_2/entPGE_2 had not been achieved with HPLC without the use of a derivatization step until Brose et al. used chiral HPLC to prove they are present in equal quantities in brain samples [49]. Because LC–MS emphasizes the need for volatile components, NP-HPLC is an often a good option. NP-HPLC can use solvents that cause concern for flammability as a result of heating elements; however, electrospray ionization (ESI) does not require heating thus eliminating this concern. HILIC separations can use a reverse-phase solvent system and achieve similar separation of eicosanoids performed by NP-HPLC. HILIC separations can have increased MS sensitivity because the effect of ion suppression is decreased. Ion-pairing reagents typically used in RP-HPLC solvent may not be required for HILIC separations of polar compounds. Although methods are reported for HILIC separation of eicosanoids [91,93] it is to our knowledge that none thus far have been specific for brain samples.

While improved LC techniques achieve separation of the metabolites of arachidonic acid, detection requires high sensitivity and specificity for a complex matrix such as the brain. An HPLC equipped with an ultraviolet (UV) detector demands that the analyte of interest must have an active chromophore. HPLC-UV relies upon the retention time as well as the UV spectrum to identify and quantify metabolites. HPLC-UV is ideal for rapid analysis and screening methods because it is simpler than more advanced techniques/detectors, yet it requires components to have chromophores. Some eicosanoids have specific chromophores, such as leukotrienes, which contain a conjugated triene; however, many eicosanoids lack active chromophores. Difficulties arise in quantifying prostanoids with a UV detector as they lack good chromophores and therefore UV detection cannot achieve necessary sensitivity levels for biological samples. UV detection is an outdated technique for quantitation of eicosanoids. In our group derivatization of eicosanoids has been coupled with fluorescence detection using RP-HPLC to determine bioactive prostaglandins, epoxyeicosatrienoic acids, dihydroxyeicosatrienoic acids and hydroxyeicosatetraenoic acids in rat brain tissue and achieved LOD's and LOQ's ranging from 2–20 to 20–70 pg on the column respectively [33]. Yue et al. derivatized eicosanoids with 2-(2,3-naphthalimino)ethyl-trifluoromethanesulphonate (NE-OTf) and determined the limit of derivatization reaction to be 0.625–8.75 pg/μL. For fluorescence detection, reagents can derivatize the free carboxyl group of fatty acid and pg detection limits can be reached. Throughout the derivatization process care must be taken to ensure reproducibility as interfering peaks from side reactions and degradation products can easily occur. As technology continues to improve, LC separations with mass spectrometry detection offer the highest sensitivity and selectivity of the metabolites of arachidonic acid. A detailed discussion of LC–MS and LC–MS/MS can be seen below.

2.2.3. Liquid chromatography–mass spectrometry, tandem mass spectrometry

The development of advanced tandem MS technology has been one of the most important advances in biomarker research in the past decade. Tandem MS/MS instruments coupled with HPLC or UPLC are capable of analyzing multiple analytes simultaneously as well as reaching sensitivity limits corresponding to those achieved by GC–MS/MS. A major advantage of LC–MS and LC–MS/MS is that derivatization is not required, which saves time and cost. Derivatization can introduce impurities that lead to decreased sensitivity in MS analysis. In HPLC-UV, when multiple analytes elute as a single peak or overlapping peaks, quantitation is not possible. Difficulties also arise because of the sensitivity and selectivity limits of UV detectors determined by the poor chromophores in arachidonic acid metabolites. The co-eluting analytes can be distinguished using LC–MS/MS by filtering specific ions. Tandem MS instruments can operate in two modes as discussed before, full-scan mode where all fragmented ions are measured or SRM/MRM mode, where selected ion pairs are monitored. The mass spectrum acquired from a scanning experiment can be a useful tool in structure elucidation and is often used prior to analysis using SRM/MRM mode to determine specific ions to monitor. LC–MS/MS offers the highest sensitivity for analysis of eicosanoid levels in biological samples when a SRM/MRM transition is monitored between an intense precursor and product ion [83], and has become indispensable since being introduced by Margalit in 1996 [86]. SRM/MRM is a principle tool used for quantitative analysis of the metabolites of arachidonic acid in which two stages of mass filtering by quadrupole 1 (Q1) and quadrupole 3 (Q3) provide absolute specificity for the metabolites. Following ionization, precursor ions are mass filtered in Q1 and fragmented by collision-induced dissociation (CID) to yield product ions that are mass filtered in Q3 and detected. Recent advances in hybrid instruments can use a

time-of-flight (TOF) or an ion trap in place of Q3 to improve the sensitivity of the product ions. Although the sensitivity of detection of metabolites is increased using tandem MS, some knowledge of the metabolites contained in the sample is required prior to analysis. When coupling LC to MS, solvent selection is important as mobile phase additives and buffers can lead to ion suppression.

Quantification of LC–MS and LC–MS/MS methods is often performed using internal standards. If stable isotope dilutions are implemented, calibration curves are created for each analyte and linearity is established over a specific range. Ideal internal standards would co-elute with the analyte of interest and be differentiated by MS analysis. However, care must be taken when selecting internal standards. Foltz and Edom demonstrated that at high concentrations co-eluting internal standards have the potential to formation multimers that can lead to non-linear calibration curves [88]. Mesaros et al. indicate that deuterium-labeled internal standards are generally separated in chromatographic analysis and therefore could experience different ion suppression as the analyte in biological samples [83]. Quantification of the linearity of standards should be performed independent of the biological matrix and later compared to a spiked sample of the biological matrix to attain the most accurate results [43] (Table 1).

LC–MS and LC–MS/MS methods have been extensively used in bioanalytical work since they combine the resolving power of LC with the detection specificity of MS. In 1986, Yergey et al. developed one of the first LC–MS methods for analysis of novel eicosanoids in brain samples [46]. Using a Beckman HPLC system coupled to an Extrel 400-2 quadrupole MS instrument with a thermospray ionization source, 15-keto-prostaglandin E_2 (15-KETO-PGE_2), hydroxyeicosatetraenoic acids (5-HETE, 12-HETE and 15-HETE) and leukotriene B_4 (LTB_4) were separated and quantitated to achieve LOD's ranging from 5 to 20 ng on the column [46]. In order to achieve desired separation and detection sensitivity, analysis needed to be performed on a single class in [46]. However, with advancing technologies, many of the limitations proposed by earlier methods are being eliminated and limits of detection are reaching the femtogram (fg) and pg range [87].

In more recent years, Masoodi and Nicolaou developed an ESI-LC-MS/MS method for analysis of 27 prostanoids and isoprostanes in biological fluids and extracts such as the brain [31]. The method demonstrates linearity in the concentration range of 1–100 pg/μL and the LOD and LOQ were 0.5–50 and 2–100 pg respectively [31]. LC–MS/MS was performed on a Waters Alliance 2695 HPLC coupled to an ESI Triple Quadrupole Quattro Ultima Mass Spectrometer (Waters, Elstree, Hertsfordshire, UK) operating in negative ion mode. LC separation was accomplished on a Gemini C18 column (5 μm, 2 × 150 mm) (Phenomenex, Macclesfield, UK) using a gradient system with mobile phase A – acetonitrile/water/glacial acetic acid (45:55:0.02, v/v/v) and mobile phase B – acetonitrile/water/glacial acetic acid (90:10:0.02, v/v/v) [31]. The collision energy and product ions were optimized for each compound. The selected transitions were PGE_2 (m/z 351 → 271), $PGF_{2\alpha}$ (m/z 353 → 193), 6-keto-$PGF_{1\alpha}$ (m/z 369 → 163), PGE_3 (m/z 349 → 269), TXB_2 (m/z 369 → 169), 8-iso-$PGF_{2\alpha}$ (m/z 353 → 193), 8-iso-15-keto $PGF_{2\alpha}$ (m/z 351 → 315) and PGD_3 (m/z 349 → 269) [31]. Masoodi et al. elected to use the same transition for the isobaric metabolites PGE_1 and PGD_1, PGE_2 and PGD_2, PGE_3 and PGD_3, PGJ_2 and Δ^{12}-PGJ_2 as well as $PGF_{2\alpha}$ and its isomer 8-iso-$PGF_{2\alpha}$ as they were separated chromatographically. Finally, detection of co-eluting prostanoids PGE_1 and PGE_2 was performed using two MRM transitions (m/z 353 → 317 and m/z 351 → 271 respectively). Masoodi et al. describe difficulties in detecting 8-iso-15-keto $PGF_{2\alpha}$ and 8-iso-PGE_2 as a result of metabolites having the same ion-pair transition. Although 8-iso-15-keto $PGF_{2\alpha}$, 8-iso-PGE_2 and PGD_2 are chromatographically resolved, 8-iso-PGE_2 co-elutes with PGE_2 and both possess the same ion-pair transition; therefore only one or the other can be detected.

Yue et al. developed one of the first MS based methods for simultaneous identification and quantification of PGs, DiHETrEs, HETEs, EETs and the parent compound AA in biological tissues, including brain tissue [43]. This is a sensitive, specific and robust LC–MS method for simultaneously analyzing parent compound AA and its COX, CYP450 and LOX pathway metabolites PGs, DiHETEs, HETEs, EETs, including $PGF_{2\alpha}$, PGE_2, PGD_2, PGJ_2, 14,15-DiHETrE, 11,12-DiHETrE, 8,9-DiHETrE, 5,6-DiHETrE, 20-HETE, 15-HETE, 12-HETE, 9-HETE, 8-HETE, 5-HETE, 14,15-EET, 11,12-EET, 8,9-EET and 5,6-EET in rat cortical brain tissue [43]. An Agilent 1100 LC/MSD was used for separation and detection of the listed metabolites. Gradient separation was performed on a Waters Symmetry® C18 column (4.6 mm × 250 mm, 5 μm) (Waters Corporation, USA) with mobile phase A and B consisting of 0.1% formic acid in deionized water and acetonitrile respectively. The mobile phase gradient consisted of 60–80% B in 30 min; 80–85% B in 5 min; 85–100% B in 1 min; 100% B for 9 min. SIM was performed in negative mode and the ions monitored were: m/z 353 ($PGF_{2\alpha}$), m/z 351 (PGD_2 and PGE_2), m/z 333 (PGJ_2), m/z 355 (PGD_2-d_4) and m/z 357 ($PGF_{2\alpha}$-d_4) from 0 to 7 min using a gain of 2; ions with m/z 337 (DiHETrEs), m/z 319 (HETEs and EETs) and m/z 327 (HETE-d_8 and EET-d_8) from 7 to 23 min using a gain of 2; and ions with m/z 303 (AA) and m/z 311 (AA-d_8) from 23 to 45 min using a gain of 1 [43]. Using ESI in negative mode the metabolites readily ionize to form carboxylate anions. The linear range of the PGs, DiHETrEs, HETEs, EETs and AA was determined to be 1–4500 pg and the levels of these metabolites in brain samples ranged from 1.24 to 3.90 pg/mg EETs, 2.16–12.14 pg/mg HETEs, 1.51–3.60 pg/mg PGE_2 and 12,512–47,727 pg/mg AA wet weight.

Kingsley and Marnett illustrate a LC–MS/MS method for analysis of neutral eicosanoids in various tissues, including brain in [48]. Although this particular method analyzes PG-like products, its applicability to related compounds is discussed. Briefly, chromatographic separation was accomplished on a Waters 2690 HPLC (Milford, MA) and SRM analyses were performed on ThermoFinnigan TSQ 7000 or Quantum Triple Quadruple MS with an ESI source (ThermoFinnigan, San Jose, CA) [48]. Specific SRM transitions are indicated for each compound with many of the PG-like compounds yielding the highest abundance precursor ion $[M+NH_4]^+$ [48]. MS analysis was performed in positive mode because the PG-like compounds do not deprotonate to generate a strong signal in negative mode like prostaglandins. The authors suggest that prostaglandins will behave similarly to the PG-like compounds because of basic similarities in their structures [48]. The LOD's of these compounds ranged from 5 to 25 femtomol (fmol) on the column. This method is applied to neutral eicosanoids and a thorough investigation on its application for the metabolites of arachidonic acid should be considered.

Golovko and Murphy improved a LC–MS/MS procedure for analysis of prostanoids in the brain [39]. Four extractions were compared to determine the optimal conditions for minimal chemical background noise in MS analysis resulting from the complex brain biological matrix. This method requires only 10 mg of brain tissue and enhanced the LOD for prostanoids 4–20-fold in brain tissue from previous methods [39]. Separation of PGE_2, PGD_2, $PGF_{2\alpha}$, TXB_2 and 6-oxo-$PGF_{1\alpha}$ was performed on a Luna C18 column (3 μm, 100 Å pore diameter, 150 × 2.0 mm; Phenomenex, Torrance, CA) using an Agilent 1100 Series LC (Agilent Technologies, Santa Clara, CA) [84]. The following gradient system was applied using 0.1% formic acid in water (mobile phase A) and acetonitrile (mobile phase B): 10% B for 2 min, 10–65% B from 2 to 8 min, 65–90% B from 15 to 20 min, 90–10% B from 35 to 37 min with an equilibration between runs for 13 min. Quantitation was optimized and performed in MRM mode on a quadrupole MS (API3000; Applied Biosystems, Foster City, CA)

Table 1
Methods for extraction and analysis of arachidonic acid metabolites.

Reference	Target analyte	Sample matrix	Extraction	Separation/ Detection	LOD, LOQ	Recovery (%)	Linearity	Chromatographic features	Mass spectrometer features	Analysis time
Abdel-Halim et al. [94]	PGE_2, $PGF_{2\alpha}$	Sprague–Dawley rat brain	Centrifuge, Liq (Ethyl acetate)	GC–MS	5 ng/g, n.r.	n.r.	n.r.	5 ft.column of 1% SE-30	Molecular ion minus trimethyl silyl ether (M^+-90), $PGF_{2\alpha,\beta}$-D_0 (494)	~3 min
Saunders et al. [37]	PGE_1, TXB_2, 6keto-$PGF_{1\alpha}$, $PGF_{2\alpha}$, AA	Bovine brain	Liq/Liq (Hexane: 2-propanol)	RP-HPLC-UV	n.r.	87.8–94.1	n.r.	Zorbex-ODS (4.6 × 250 mm), λ_{ABS} −192 nm	N/A	30 min
Ogorochi et al. [58]	PGE_2, PGD_2, $PGF_{2\alpha}$, 6-KETO-$PGF_{2\alpha}$	Human brain	SPE (C18 Sep-PAK)	GC–MS	n.r.	n.r.	n.r.	Thermostable cross-linked OV-1 fused silica capillary column (0.31 × 0.31 mm)	PGE_2 and PGD_2 (552.3540), $PGF_{2\alpha}$ (625.4140), 6-KETO-$PGF_{2\alpha}$ (670.4354)	11 min
Yergey et al. [46]	15-HETE, 12-HETE, 5-HETE, LTB_4, 15-KETO-PGE_2	Rat brain	SPE (C18 Sep-PAK)	RP-HPLC-(Positive Ion)TSP-MS	5–20 (ng on-column), n.r.	n.r.	n.r.	Du Pont (4.6 × 250 mm), Zorbex-ODS (5 μm, 4.6 × 250 mm)	Scanning (110–510 Da)	33 min
Birkle et al. [72]	PGE_2, PGD_2, TXB_2, LTB_4, 6keto-$PGF_{1\alpha}$, $PGF_{2\alpha}$, 15-HETE, 12-HETE, 5-HETE	Rat brain	Liq/Liq (chloro-form:methanol), liq/liq (hexane:2-propanol)	RP-HPLC-flow scintillation detection	n.r.	93	n.r.	RadialPAK pBondapak C18 column (10 μm, 8 × 100 mm)	N/A	100 min
Yasaka et al. [44]	AA	Mouse brain	Liq (Single-Step Methanol)	RP-HPLC-FLD	4 fmol, n.r.	95	n.r.	Chemcosorb 5C8 (5 μm, 4.6 × 150 mm), λ_{EX} – 259 nm and λ_{EM} – 394 nm	N/A	20 min
Amruthesh et al. [75]	PGE_2, 20-OH-LTB_4, LTB_4, LXA_4, 6keto-$PGF_{1\alpha}$, 14,15-DiHETrE, 8,9-DiHETrE, 5,6-DiHETrE, 15-HETE, 12-HETE, 5-HETE, 14,15-EET, 8,9-EET, 5,6-EET, AA	Male ICR mouse brain	Liq (ethyl acetate)	RP-HPLC-flow scintillation detection, NP-HPLC, GC-EI-MS, GC-EICI-MS	n.r.	n.r.	n.r.	Radial-PAK A C18 (4 μm, 8 × 100 mm), pPorasil Radial-Pak A (4 μm, 8 × 100 mm), Hewlett-Packard Ultra-1 cross-linked methyl silicone capillary column (25 m)	Methyl ester trimethylsilyl ether derivatives of 15-HETE, 12-HETE, 5-HETE were analyzed by SIM on predominant ions, PFB esters of 14,15-EET, 5,6-EET were analyzed (319, 301)	120, 40, 11.67, 16.67 min
Wiswedel et al. [79]	12-HHT, 2-HETE, 3-HETE, 5-HETE, 8,9-HETE, 11,12-HETE, 15-HETE, 8-iso-$PGF_{2\alpha}$, $9_\alpha,11_\beta$-$PGF_{2\alpha}$, $9_\alpha,11_\alpha$-$PGF_{2\alpha}$	Rat brain mitochondria	SPE (C18, NH2)	GC-NICI-MS	~fmol level	n.r.	n.r.	DB 5-MS column (30 m × 0.25 mm i.d.; 0.25 mm film thickness)	12-HHT (SIM of carboxylate anion $[M-181]^-$ at m/z 357) 2-HETE, 3-HETE, 5-HETE, 8,9-HETE, 11,12-HETE, 15-HETE (SIM of carboxylate anion $[M-181]^-$ at m/z 399) 8-iso-$PGF_{2\alpha}$, $9_\alpha,11_\beta$-$PGF_{2\alpha}$, $9_\alpha,11_\alpha$-$PGF_{2\alpha}$ (SIM of carboxylate anion $[M-181]^-$ at m/z 569)	30, 32.2 min

Table 1 (*Continued*)

Reference	Target analyte	Sample matrix	Extraction	Separation/ Detection	LOD, LOQ	Recovery (%)	Linearity	Chromatographic features	Mass spectrometer features	Analysis time
Rosenberger et al. [84]	PGE_2, PGD_2, TXB_2, $PGF_{1\alpha}$, $PGF_{2\alpha}$	Rat brain	Liq/Liq (n-Hexane/2-Propanol)	GC-FID	n.r.	n.r.	n.r.	SP 2330 capillary column (30 m × 0.32 mm i.d.)	N/A	20 min
Yue et al. [33]	$PGF_{2\alpha}$, PGE_2, PGD_2, PGJ_2, 14,15-DiHETrE, 11,12-DiHETrE, 8,9-DiHETrE, 20-HETE, 15-HETE, 12-HETE, 14,15-EET, 11,12-EET, 8,9-EET, AA	Male Sprague–Dawley rat brain	SPE (Oasis®HLB)	RP-HPLC-FLD, RP-HPLC-(negative ion)ESI-MS	2–20, 20–70 (pg on-column)	72.7–117.2	70–2000 pg	Symmetry® C18 column (5 μm, 2.1 × 150 mm), λ_{EX} – 260 nm and λ_{EM} – 396 nm	$PGF_{2\alpha}$ (646 Da), PGE_2 and PGD_2 (644 Da), PGJ_2 (626 Da), 14,15-DiHETrE (631 Da), 11,12-DiHETrE (631 Da), 8,9-DiHETrE (631 Da), 20-HETE (612 Da), 15-HETE (612 Da), 12-HETE (612 Da), 14,15-EET (612 Da), 11,12-EET (612 Da), 8,9-EET (612 Da), AA (596 Da)	100 min
Masoodi et al. [31]	PGE_1, PGD_1, PGE_2, PGD_2, $PGF_{2\alpha}$, 8-iso-$PGF_{2\alpha}$, 6-keto-$PGF_{1\alpha}$, PGE_3, PGD_3, TXB_2, TXB_3, 8-iso-15-keto $PGF_{2\alpha}$, PGJ_2, Δ^{12}-PGJ_2, PGB_2, 15-deoxy-Δ^{12},14-PGJ_2, $PGF_{3\alpha}$, 8-iso-PGE_2, 8-iso-15-keto PGE_2, 13,14-dihydro PGE_1, 13,14-dihydro-15-keto PGE_1, 13,14-dihydro-$PGF_{1\alpha}$, 13,14-dihydro-15-keto $PGF_{1\alpha}$, 13,14-dihydro-15-keto PGE_2, 13,14-dihydro $PGF_{2\alpha}$, 13,14-dihydro-15-keto $PGF_{2\alpha}$	Male Wistar rat brain	SPE (Phenomenex C18-E)	RP-HPLC-(Negative Ion)ESI-MS/MS	0.5–50, 2–100 (pg on-column)	83–116	1–100 pg/μL	Phenomenex Gemini C18 (5 μm, 2.0 × 150 mm)	PGE_1 and PGD_1 (353 → 317), PGE_2 and PGD_2 (351 → 271), $PGF_{2\alpha}$ and 8-iso-$PGF_{2\alpha}$ (353 → 193), 6-keto-$PGF_{1\alpha}$ (369 → 163), PGE_3 and PGD_3 (349 → 269), TXB_2 (369 → 169), TXB_3 (367 → 169), 8-iso-15-keto $PGF_{2\alpha}$ (351 → 315), PGJ_2 and Δ^{12}-PGJ_2 (353 → 193), PGB_2 (333 → 175), 15-deoxy-$\Delta^{12,14}$-PGJ_2 (315 → 271), $PGF_{3\alpha}$ (351 → 193), 8-iso-PGE_2 (351 → 315), 8-iso-15-keto PGE_2 (349 → 113), 13,14-dihydro PGE_1 (355 → 337), 13,14-dihydro-15-keto PGE_1 (353 → 335), 13,14-dihydro-$PGF_{1\alpha}$ (357 → 113), 13,14-dihydro-15-keto $PGF_{1\alpha}$ (355 → 193), 13,14-dihydro-15-keto PGE_2 (351 → 333), 13,14-dihydro $PGF_{2\alpha}$ (355 → 311), 13,14-dihydro-15-keto $PGF_{2\alpha}$ (353 → 113)	30 min

Yue et al. [43]	$PGF_{2\alpha}$, PGE_2, PGD_2, PGJ_2, 14,15-DiHETrE, 11,12-DiHETrE, 8,9-DiHETrE, 5,6-DiHETrE, 20-HETE, 15-HETE, 12-HETE, 9-HETE, 8-HETE, 5-HETE, 14,15-EET, 11,12-EET, 8,9-EET, 5,6-EET	Male Sprague–Dawley rat brain	SPE (Oasis®HLB)	RP-HPLC-(Negative Ion)ESI-MS	n.r., 2–20 (pg on-column)	54.84–99.56	2–2400 pg	Symmetry® C18 column (3.5 μm, 2.1 × 150 mm)	$PGF_{2\alpha}$ (353), PGE_2 and PGD_2 (351), PGJ_2 (333), 14,15-DiHETrE (337), 11,12-DiHETrE (337), 8,9-DiHETrE (337), 5,6-DiHETrE (337), 20-HETE (319), 15-HETE (319), 12-HETE (319), 9-HETE (319), 8-HETE (319), 5-HETE (319), 14,15-EET (319), 11,12-EET (319), 8,9-EET (319), 5,6-EET (319)	35 min
Masoodi et al. [54]	15-HETE, 12-HETE, 11-HETE, 9-HETE, 8-HETE, 5-HETE, LTB_4	Male Wistar rat brain	SPE (Phenomenex C18-E)	RP-HPLC-(negative ion)ESI-MS/MS	10–20, 20–50 (pg on-column)	76–122	1–100 pg/μL	Luna C18 column (5 μm, 2.0 × 150 mm)	15-HETE (351.2 → 175), 12-HETE (351.2 → 179), 11-HETE (351.2 → 167), 9-HETE (351.2 → 155), 8-HETE (351.2 → 123), 5-HETE (319 → 115), LTB4 (335 → 195)	28 min
Golovko et al. [39]	PGD_2, PGE_2, TXB_2, 6-oxo-$PGF_{1\alpha}$, $PGF_{2\alpha}$	Mouse brain	Acetone, followed by Liq/Liq purification	RP-HPLC-(negative ion)ESI-MS/MS	0.6–3.3 (pg on-column), n.r.	85–95	n.r.	Luna C18 (3 μm, 100 Å, 2.0 × 150 mm)	PGD_2 and PGE_2 (351.2 → 271.5), TXB_2 (369.2 → 169.2), 6-oxo-$PGF_{1\alpha}$ (369.2 → 163.4), $PGF_{2\alpha}$ (353.3 → 193.2)	17 min
Farias et al. [66]	AA, DHA, DPA, E_2/D_2-IsoPs, $PGF_{2\alpha}$, 8-iso-$PGF_{2\alpha}$, TXB_2, 5-HETE, 5-oxo-ETE, 12-HETE, LTC_4, LTB_4	Male Fischer CDF (F-344)/CrlBR rat brain	SPE (Strata C18-E), SPE (Oasis®HLB)-IsoPs	RP-HPLC-(Negative Ion)ESI-MS/MS	n.r., 0.16–1.15 pmol	60–70	n.r.	Colombus RP-C18 column (5 μm, 1 × 150 mm)	AA (303 → 205); DHA (327 → 283); DPA (329 → 285); E_2/D_2-IsoPs (351 → 271), (351 → 233), (351 → 333), (351 → 315); $PGF_{2\alpha}$ and 8-iso-$PGF_{2\alpha}$ (353 → 193); TXB_2 (369 → 169); 5-HETE (319 → 115); 5-oxo-ETE (317 → 13); 12-HETE (319 → 179); LTC_4 (624 → 272); LTB_4 (335 → 195)	45 min
Milatovic et al. [81]	F_2-isoprostanes, AA	Neuronal tissue	Liq/Liq (Folch Method) SPE (Sep-Pak Plus C18 cartridge)	GC-NICI-MS	~5 pg, (low pg range)	n.r.	n.r.	DB1701 fused silica capillary (15 m, 0.25-mm diameter, 0.25-μm film thickness)	F_2-isoprostanes (569)	~15 min

Table 1 (*Continued*)

Reference	Target analyte	Sample matrix	Extraction	Separation/ Detection	LOD, LOQ	Recovery (%)	Linearity	Chromatographic features	Mass spectrometer features	Analysis time
Miller et al. [52]	20-HETE, 15-HETE, 12-HETE, 14,15-EET, 11,12-EET, 8,9-EET, 14,15-DiHETrE, 11,12-DiHETrE, 8,9-DiHETrE, 5,6-DiHETrE	Sprague–Dawley rat brain cortical tissue	SPE (Oasis®HLB)	RP-UPLC-(negative ion)ESI-MS/MS	n.r., 0.208 ng/μL	72.73–94.11	0.208–33.3 ng/μL	ACQUITY BEH C18 column (1.7 μm, 2.1 × 100 mm)	20-HETE (319 → 245,289), 15-HETE (319 → 219), 12-HETE (319 → 179), 14,15-EET (319 → 219), 11,12-EET (319 → 167), 8,9-EET (319 → 127), 14,15-DiHETrE (337 → 207), 11,12-DiHETrE (337 → 167), 8,9-DiHETrE (337 → 127), 5,6-DiHETrE (337 → 145)	4.8 min
Brose et al. [49]	PGD_2, PGE_2, 8-isoPGE_2, ent-PGE_2, 11β-PGE_2, 5trans-PGE_2, 15R-PGD_2	Mouse brain (Ischemic Brain Tissue)	Acetone using Liq/Liq	RP-HPLC-(Negative Ion)ESI-MS/MS, Chiral-HPLC-(Negative Ion)ESI-MS/MS	0.3 ± 0.1 (pg on-column)	~90	1 pg–100 ng on-column	Luna C-18 (3 μm, 100 Å, 2.0 × 150 mm), 2 Tandem Chiral Lux Amylose 2 columns (3 μm, 100 Å, 2.0 × 150 mm)	PGD_2 (351.2 → 189.5), PGE_2 (351.2 → 189.5), 8-isoPGE_2 (351.2 → 189.5), ent-PGE_2 (351.2 → 189.5), 11β-PGE_2 (351.2 → 189.5), 5 trans-PGE_2 (351.2 → 189.5), 15R-PGD_2 (351.2 → 189.5)	65 min
Dumlao et al. [45]	141 eicosanoid metabolites generated from cycloxygenase, lipoxygenase, cytochrome P450 enzymes and non-enzymatic pathways	Application in various tissue, cerebral spinal fluid presented	SPE (Strata-X polymerized, Phenomenex)	RP-HPLC-(Negative Ion)ESI-MS/MS	0.1–1, 1–10 pg	n.r.	n.r.	Synergi RP-C18 column (2.1 × 250 mm)	See reference for specific transitions and optimized parameters for 141 eicosanoid metabolites	25 min
Yang et al. [77]	AA	Male Wistar Rats (traumatic brain injury)	Liq (methanol)	GC-EI-MS	n.r.	n.r.	n.r.	30-m DB-5 column (30 m × 250 μm i.d., 0.25 μm film thickness)	Full Scan (30–550)	59 min
Strauss et al. [62]	15-HETE, 12-HETE, 11-HETE, 8-HETE, 5-HETE, 14,15-EET, 11,12-EET, 8,9-EET, 5,6-EET	Mouse brain	SPE (Oasis®HLB)	RP-UPLC-(Negative Ion)-MS/MS	n.r., n.r.	25–50	1–125 pg	ACQUITY BEH C18 column (1.7 μm, 2.1 × 100 mm)	Employed multiple reaction mode (MRM), exact MRM transitions not listed	8 min
Brose et al. [42]	PGD_2, PGE_2, 8-isoPGE_2, 15R-PGE_2, ent-PGE_2, Δ12-PGD_2, 11β-PGE_2, 5trans-PGE_2, 15R-PGD_2	Mouse Brain (Ischemic Brain Tissue)	Liq (Single-step Methanol)	RP-UPLC-(Negative Ion)ESI-MS/MS	n.r., 1 ± 0.5 (pg on-column)	92.9 ± 12.1 –96.7 ± 9.9	1 pg–50 ng	ACQUITY UPLC HSS T3 column (1.8 μm, 100 Å, 2.1 × 150 mm)	PGE_2 (351.2171 → 189.1279), PGE_2-d_4 (355.2391 → 275.2391)	4.5 min

Tajima et al. [50]	31 arachidonic acid metabolites	Mouse brain (APP/tau Mice)	Liq/Liq (Bligh and Dyer Method)	RP-UPLC-(Negative Ion)ESI-MS/MS	n.r.	n.r.	n.r.	ACQUITY BEH C18 column (1.7 μm, 1.7 × 150 mm)	n.r.	50 min
Liu et al. [70]	PGJ_2, $\Delta^{12}PGJ_2$, 15d-PGJ_2, 15d-PGD_2, PGD_2	Male Sprague–Dawley rat brain	SPE (Oasis®HLB)	RP-UPLC-(Negative Ion)-MS/MS	n.r.	n.r.	n.r.	ACQUITY BEH C18 column (1.7 μm, 2.1 × 100 mm)	PGJ_2 and $\Delta^{12}PGJ_2$ (333 → 271), 15d-PGJ_2 (315 → 271), 15d-PGD_2 (333 → 271), PGD_2 (351 → 271)	n.r.

using a turbo-ion-spray ionization source and the following MRM transitions were monitored: PGE_2 and PGD_2 (m/z 351.2 → 271.5), $PGF_{2\alpha}$ (m/z 353.3 → 309.3), TXB_2 (m/z 369.2 → 169.2) and 6-oxo-$PGF_{1\alpha}$ (m/z 369.2 → 163.4) [84]. The LOD's reported in picograms on the column were PGE_2/PGD_2 (0.5 ± 0.1), TXB_2 (0.7 ± 0.2), $PGF_{2\alpha}$ (1.2 ± 0.1) and 6-oxo-$PGF_{1\alpha}$ (2.1 ± 0.5). Additionally, the mass in nanograms/gram wet weight for 20 mg brain samples was determined to be PGE_2/PGD_2 (0.1 ± 0.0), TXB_2 (0.1 ± 0.0), $PGF_{2\alpha}$ (0.1 ± 0.0) and 6-oxo-$PGF_{1\alpha}$ (0.3 ± 0.1) [84]. This method effectively separated all the prostaglandins and isoprostanes of interest except PGE_2 and 8-iso-PGE_2, which co-eluted. Results were consistent with those reported by Masoodi et al. and who performed analysis of isoprostanes and prostaglandins using similar chromatographic parameters [31,85].

Biomarker discovery for application in pharmacological approaches is becoming increasingly important. Masoodi et al. present a LC–MS/MS system for analysis of seven arachidonic acid metabolites in brain samples of Wistar rats [54]. This approach can be used in combination with [31] for various lipidomic applications. One of the challenges of analysis of arachidonic acid metabolites is achieving suitable sensitivity in detection. Single quadrupole MS instruments offer increased sensitivity over diode array detection, but are limited by certain factors such as chromatographic separation. MRM mode allows for increased sensitivity of detection by increasing the specificity for each metabolite. Analysis of 5-HETE, 8-HETE, 9-HETE, 11-HETE, 12-HETE, 15-HETE, LTB_4 and 12S-HETE-*d8* was performed using a Luna C18 column (5 μm, 2 mm × 150 mm; Phenomenex, Macclesfield, UK) on a Waters Alliance 2695 HPLC system coupled to a Triple Quadrupole Quattro Ultima Mass Spectrometer (Waters, Elstree, Hertsfordshire, UK). Chromatographic separation was achieved using isocratic conditions at 95:5 (A:B) consisting of mobile phase A (methanol/water/glacial acetic acid, 80:20:0/02, v/v/v) and mobile phase B (acetonitrile/water/glacial acetic acid, 45:55:0.02, v/v/v). MRM transitions were optimized for the highest abundance product ions and the ones documented by this method were: 5-HETE (m/z 319 → 115), 8-HETE (m/z 319 → 155), 9-HETE (m/z 319 → 123), 11-HETE (m/z 319 → 167), 12-HETE (m/z 319 → 179), 15-HETE (m/z 319 → 175) and LTB_4 (m/z 335 → 195) [54]. Isobaric 8-HETE and 12-HETE were not able to be resolved chromatographically; however, because they possess structure specific fragment ions simultaneous detection is possible in this method. LOD's and LOQ's are reported ranging from 10 to 20 pg on the column and 20–50 pg on the column respectively. The method of Masoodi et al. demonstrate adequate sensitivity for detection of arachidonic acid metabolites in brain samples.

Although HPLC–MS/MS has been at the forefront of quantitating biomarkers in biological samples, currently UPLC–MS/MS is becoming the frontrunner as it overcomes limitations of traditional HPLC such as low flow rate and large injection volumes. Miller et al. developed a high throughput UPLC–MS/MS method for quantitation of hydroxyeicosatetraenoic acid (HETE), dihydroxyeicosatrienoic acid (DiHETrE), and epoxyeicosatrienoic acid (EET) metabolites of arachidonic acid in rat brain cortical tissues with a potential application in assessing the risk of symptomatic cerebral vasospasm (SV) in subarachnoid hemorrhage (SAH) patients [52]. UPLC methods are designed to utilize efficient separation to reduce run times. Chromatographic separation was performed on an ACQUITY UPLC (Waters, Milford, MA) using a BEH C18 column (1.7 μm, 2.1 mm × 100 mm) (Waters, Milford, MA) with mobile phases consisting of A – 0.005% acetic acid, 5% acetonitrile in deionized water and B – 0.005% acetic acid in acetonitrile [52]. The following parameters were used for the mobile phase gradient: 0–4 min (35–70% B), 4–4.5 min (70–95% B) and 4.5–4.8 min (95% B). Quantitation was executed in negative SRM mode using a TSQ Quantum Ultra (Thermo Fisher Scientific, San Jose, CA) triple quadrupole

mass spectrometer coupled with heated electrospray ionization (HESI) [52]. The MS parameters were optimized to achieve the highest $[M-H]^-$ ion abundance and the following *m/z* transitions were used: 20-HETE (319 → 245), 15-HETE (319 → 219), 12-HETE (319 → 179), 14,15-EET (319 → 219), 11,12-EET (319 → 167), 8,9-EET (319 → 127), 14,15-DiHETrE (337 → 207), 11,12-DiHETrE (337 → 167), 8,9-DiHETrE (337 → 127), 5,6-DiHETrE (337 → 145) and 20-HETE-*d6* (319 → 251) respectively. The LOQ for all the metabolites studied was 0.208 ng/mL and samples from rat brain cortex tissue demonstrated levels of 20-HETE, 15-HETE, 12-HETE, 8,9-EET 14,15-DiHETrE and 11,12-DiHETrE ranging from 0.57 to 23.99 pmol/g wet tissue. The matrix effect was monitored by determining the coefficient of variance (CV) for the peak areas of each metabolite at low and high concentration levels. Both levels demonstrated CV's lower than 15%, exemplifying the consistency of the assay. Miller et al. report a reliable method for analysis of 10 arachidonic acid metabolites in several tissues, including the brain and demonstrates advantages over other methods [68,69] used for analysis of similar metabolites, such as shortened run times from 47 and 31 min to 6.4 min.

With advancing MS technology the development of high-throughput methods is escalating. Tandem MS methods are limited by the number of transitions that can be monitored [83]. Overcoming this issue, a targeted comprehensive lipidomic analysis by separation simplification (CLASS) approach is presented by Dumlao et al. in which an eicosanoid methodology demonstrates the ability to monitor and quantitate 171 scheduled multiple reaction monitoring (sMRM) pairs [45]. A MRM pair correlated to a specific LC retention time is referred to as an sMRM pair [45]. The principal constraint of this method is the amount of standard metabolites available. As more standards become accessible the efficiency and application of this method will be increased. The present method can be applied to various tissues, but is utilized here for analysis of cerebral spinal fluid (CSF). Chromatographic separation was performed on a Synergi RP-C18 column (2.1 × 250 mm, Phenomenex, CA) using mobile phases A and B, comprised of water/acetonitrile/acetic acid (70:30:0.02, v/v/v) and acetonitrile/isopropyl alcohol (50:50, v/v) respectively [45]. The mobile phase gradient applied was 1–3 min (0–25% B), 3–11 min (25–45% B), 11–13 min (45–60% B), 13–18 min (60–75% B), 18–18.5 min (75–90% B), 18.5–20 min (90% B), 20–21 min (90–0% B) and 21–25 min (0% B) [45]. Tandem MS analysis in sMRM mode using an ABI/Sciex (Foster City, CA) 4000 QTRAP hybrid, triple quadrupole linear ion trap mass spectrometer with a Turbo V ion source achieved LOD's ranging from 0.1 to 1 pg [45]. Dumlao et al. established for each eicosanoid the precursor ion, product ion, LOD, declustering potential and collisional energy applied [45]. It was evident that analyzing 171 metabolites does not allow for complete chromatographic separation and detection by MS became difficult, as many metabolites had only slight modifications in their molecular structure and produced the same product ions. However, application of sMRM mode allows for detection of similar metabolites by using associated retention times. The sMRM approach demonstrated its detection effectiveness when analyzing a cluster of metabolites with overlapping chromatographic peaks in Dumlao et al. [45]. This method takes advantage of advanced mass spectrometer algorithms, optimizes data collection and is anticipated as a starting point for future eicosanoid research of large data sets [45].

The work of Brose et al. demonstrates a single step extraction UPLC–MS/MS method for analysis of the E_2/D_2 series prostaglandins and isoprostanes in brain tissue [42]. This UPLC–MS/MS method improves an HPLC–MS/MS method previously reported [49]. Lengthy separation times result in broad chromatographic peaks that decrease detection limits and in turn limit sensitivity in MS analysis. The UPLC–MS/MS improves the separation time of major isobaric iso-PGE_2 of the HPLC method from 1 h to 4 min. Utilizing a single solvent (methanol) for extraction purposes, proved to decrease chemical background noise and increase MS sensitivity. Separation of PGE_2 and PGD_2 was executed on an ACQUITY HSS T3 column (1.8 μM, 100 Å pore diameter, 2.1 × 150 mm, Waters, Milford, MA) with a Waters Acquity UPLC (Waters, Milford, MA). A simple aqueous solvent system consisting of 0.1% formic acid in water (mobile phase A) and acetonitrile (mobile phase B) was used to perform the following gradient: 39% B for 0.5 min, 39–40.5% B over the next 6.88 min, 40.5–98% B over the next 0.2 min and 98% B for 2 min [42]. Separation of the PGE_2 and PGD_2 occurred within 4 min making this an extremely fast method. An issue Brose exposed when using methanol as a sole means of extraction was that hydrophobic lipids would not elute from the column and therefore extensive cleaning is required if brain samples are frequently analyzed [42]. Quantification of PGE_2 and PGD_2 was performed using a Quadrupole Time-of-Flight Mass Spectrometer (Q-TOF, Synapt G2-S, Waters, Milford, MA) monitoring the MRM transition (*m/z* 351 → 189). Linearity was established for the concentration range of 1 pg–50 ng on the column for the analytes, and the LOQ was determined to be 1 ± 0.5 pg [42]. This method was applied to quantify endogenous levels of E_2/D_2 prostaglandins and isoprostanes in ischemic brain tissue and determined PGD_2 and PGE_2 to be present at 1.02 ± 0.06 and 5.07 ± 0.33 ng/gww respectively. Additionally it increases sensitivity of analysis while decreasing time and cost of analysis, and is proven effective for detection of prostaglandins. Although Brose et al. present a desirable UPLC–MS/MS method for analysis of prostaglandins and isoprostanes in brain tissue, it falls short on achieving the separation of PGE_2 and 15R-PGE_2 as well as PGE_2 and ent-PGE_2. Chromatographic resolution of PGE_2 and ent-PGE_2 is important, as it has been suggested that analysis of their ratio may lead to demonstrating the origin of PGE_2 in biological samples [49]. Chiral separation of PGE_2 and ent-PGE_2 was performed on two Lux Amylose2 columns (3 μm, 100 Å pore diameter, 150 × 2.0 mm; Phenomenex) connected in series using an acetonitrile/water/formic acid gradient and quantification was accomplished in MRM mode, monitoring the precursor and product ions *m/z* 351.2 and 189.5 respectively. Brose et al. optimized MS/MS parameters to achieve the highest selectivity for 15-series isoPGE_2 [49]. Product ions produced from PGE_2 are generated by a water and carboxyl group loss. As a result, product ions 333, 315 and 271 are not specific toward 15-series isoPGE_2. The 189.5 product ion is generated by a side chain loss ($-C_6H_{10}$, $-CO_2$, $-H_2O \times 2$), which allows for selective quantitation for 15-series isoPG and eliminates up to 48 possible isoforms [49]. Brose et al. determined that the 189.5 product ion demonstrates high specificity and selectivity than previously published methods using 351 → 271 transition [49]. The detection limits reported were 0.3 ± 0.1 pg and the method was applied to analyze mouse brain fixed with head-focused microwave irradiation before and after global ischemia [49]. Advances in chiral UPLC will significantly reduce the analysis time of the reported method.

Lipidomic analysis of brain tissue from APP/tau mice and control mice at pre-symptomatic and post-symptomatic phases was performed using RPLC-ESI-Triple Quadrupole MS/MS in [50]. Separation and quantitation of 31 arachidonic acid metabolites was performed on an ACQUITY UPLC (Waters) system using a ACQUITY BEH C18 column (1.7 mm × 150 mm, 1.7 μm, Waters) coupled to a 5500 Quadrupole-Linear Ion Trap Hybrid MS (QTRAP) (AB Sciex, Framingham, MA, USA) [50]. Mobile phases A and B were composed of water/acetate (100:01, v/v) and acetonitrile/methanol (4:1, v/v) respectively. The following gradient was applied for measurement of the metabolites: 0–5 min (27–50% B), 5–35 min (50–80% B), 35–40 min (80–100% B) and 40–50 min (100% B). Tajima et al. adjusted the flow rate from 50 μL/min (0–35 min), 50–100 μL/min (35–40 min) and finally 100 μL/min (40–50 min) to improve the

resolution of the later eluting compounds. This particular UPLC method is employed to analyze a total of 62 metabolites (31 metabolites of arachidonic acid) in 50 min. Reducing the flow to 50 μL/min in an UPLC instrument can drastically change the efficiency of separation as the number of theoretical plates is affected directly by the mobile phase velocity. At extremely low velocity the number of theoretical plates is the highest, which is likely a reason the flow rate of 50 μL/min was chosen; however, the longer a compound spends on the column the broader its chromatographic peaks will get. Furthermore, as discussed before, broad chromatographic peaks will decrease the sensitivity of the detector. Utilization of the advances UPLC offers is not readily seen in [50]. Tajima et al. monitored fold changes (ratio of APP/tau to wild-type) in PGD_2, TXB_2, 12-HHT, 12-HETE, 15-HETE, 11,12-EpETrE and 14,15-EpETrE and observed a significant decrease in PGD_2 and 15-HETE over 10 months, no specific concentrations and LOD's are reported [50].

2.2.4. Ultra high pressure liquid chromatography

Separation of multiple metabolites is detailed and often requires long chromatographic analysis time as a result of the limitation of flow rate applied in HPLC. Extensive time spent on the column can lead to decreased sensitivity in detection due to broad chromatographic peak shapes. UPLC increases resolution, speed and sensitivity for analysis of metabolites. The particle size of HPLC columns has gradually decreased from 10 to 3 μm the 1970–1990's, thus increasing separation efficiency. In a survey comparing column usage from 1997 to 2007 a shift in interest is seen from HPLC columns of 5 μm particle size to UPLC columns of <2 μm particle size [71]. A reduction in particle size to 1.7 μm (UPLC columns) has demonstrated a further enhancement in the efficiency of separation as described by Van Deemter's equations. At low linear velocities columns will experience reduced performance; however, if the particle size is decreased an improvement can be seen in column performance. Additionally, decreased particle sizes can demonstrate less reduction in column performance at increased linear velocities as compared to larger particle sizes. Band broadening is a consequence of long analysis times often used in eicosanoid analysis. Broader peaks decrease the signal-to-noise ratio consequently decreasing the sensitivity of the detector. Decreasing the height equivalent theoretical plates (HETP) by decreasing particle size will decrease band broadening. As a result of increased speed of analysis, UPLC lowers use of solvent, thus decreasing cost and making the technique more environmentally friendly, or "green". Additionally, increased speed offers a higher throughput of analysis. With advancements in technology, UPLC utilizes the ability to sustain pressures of 15,000 psi (1000 bar) compared to 5802 psi (400 bar) for traditional HPLC. Additionally UPLC columns are packed with fully porous particles or particles that contain a solid core. The solid core improves peak shape as solvents and analytes are no longer able to penetrate into particle pores, hence reducing axial diffusion [67]. Kortz et al. improved the resolution of PGD_2 and PGE_2 from 1.4 to 2.9 and reduced the analysis time in half using a Kinetex core–shell column rather than a standard C18 column with fully porous particles [92]. However, difficulties arise when analyzing various matrices with solid core particles, and fully porous particles may be favored for increased permeability [67]. Brose et al. developed a UPLC–MS/MS method to analyze the E_2/D_2 prostaglandins and isoprostanes from mouse brains in which separation was achieved within 4 min for the major species and with five times narrower chromatographic peaks compared to traditional HPLC methods [42]. A single step extraction was employed and the LOQ for this UPLC–MS/MS method was 1 ± 0.5 pg on the column. Enhancing peak shape allows for smaller injection volumes, and smaller injection volumes can result in better resolution in the mass spectrometer. However, in less concentrated solutions larger injection volumes are often needed and Brose et al. reported a change in peak shape when larger volumes of analyte were injected [42]. Similarly Miller et al. reported results for analysis of 8,9-DiHETrE, 20- HETE, 15-HETE, 12-HETE in cerebral spinal fluid and 12-, 15-, and 20-HETE, 11,12- and 14,15-DiHETrE and 8,9-EET in rat brain cortical tissue using UPLC–MS/MS that were comparable to Yue et al., Yoshida et al. and Nueman et al. with a shortened run time of 6.2 min verses run times averaging >30 min [43,52,68,69]. The concentrations of these metabolites were linear within the range of 0.208–33.3 ng/ml with a LOQ for all metabolites of 0.208 ng/ml [52]. Cerebral cortex levels of EETs (5,6-EET, 8,9-EET, 11,12-EET and 14,15-EET) and HETEs (5-HETE, 8-HETE, 11-HETE, 12-HETE and 15-HETE) were reported ranging from 1 to 49 pg/mg protein in [62]. Yue et al. demonstrate detection of DiHETrE metabolites in [43]. This method [52] alludes to the difficulty of detecting DiHETrEs as a result of their response being below the LOQ and suggests further research should be investigated. Lastly, the concentration of cyclopentenone prostaglandins (CyPGs) after temporary focal ischemia was determined by UPLC–MS/MS in SRM mode to range from 1 nM to 5 μM depending on the metabolite in [70]. It can be anticipated that UPLC will become used more frequently for analysis of arachidonic acid metabolites. See Table 1 for a list of methods for extraction and analysis of arachidonic acid metabolites.

3. Conclusion

Eicosanoids are potent lipid mediators of inflammation and are known to play an important role in numerous pathophysiological processes. For that reason they are actively researched for their roles in various diseases and applications. The desire for dependable analytical techniques that quantify picogram amounts or lower of these metabolites in various biological samples is escalating. In regards to analysis of the brain tissue, extraction of arachidonic acid metabolites is a particularly challenging issue as a result of the complex matrix. Solvent and SPE extractions have been discussed that have proven to increase selectivity and sensitivity. Structure determination and quantitation of arachidonic acid metabolites in brain tissue can be regularly performed by GC–MS and GC–MS/MS, but is complicated by sample preparation, analysis time and cost; therefore, focus has shifted toward liquid chromatography. Applications of LC instruments equipped with UV detectors are limited for eicosanoids as a result of the requirement of chromophores. Quantitation becomes difficult at levels required for analysis of brain samples. Fluorescence detection is complicated by sample preparation, but can offer improved results in comparison to UV detectors. The development of LC–MS and LC–MS/MS methods has transformed the approach to analyze arachidonic acid metabolites. LC–MS and LC–MS/MS do not require derivatization, thus reducing time and cost. In comparison to GC methods, LC–MS and LC–MS/MS methods are more versatile and often easier to execute. Tandem MS/MS instruments offer the highest sensitivity for analysis of arachidonic acid metabolites in brain samples. Tandem MS/MS instruments operating in SRM/MRM allow for analysis of similar eluting compounds as a result of being capable of selecting specific ion pairs. Finally, UPLC offers increased resolution, speed and sensitivity for analysis of metabolites. Although these instruments are costly, the potential for high throughput methods has been demonstrated. Future advances in instrumentation will allow for higher throughput methods that are simpler to operate and offer improved selectivity, sensitivity and lower detection limits.

Acknowledgments

The authors would like to thank Dr. Bruce Weber and Tiffany Gilles for their critical reviews of this manuscript.

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